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The Distributional Kinetics of Ni^{2+} in the Rat Lung

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16. ABSTRACT

The kinetics of removal of Ni^{2} from the airways of the rat lung was studied in vivo and ex vivo. The uptake of Ni^{2} from the vasculature was investigated ex vivo. For ex vivo studies, the isolated, venilated, and perfused lung (IVPL) was prepared using negative pressure ventilation and was perfused with Krebs-Henseleit buffer solution at 37°C. Rates of removal of Ni^{2} from the airways were determined following intratracheal instillation of 1, 10, or 127 nmol $^{63}NiCl_2$ in 100 µl of isotonic sucrose. The Ni^{2} remaining in the lung was calculated from the $^{63}Ni^{2+}$ activity which appeared in the perfusate. Ex vivo studies revealed a concentration dependent alteration in lung permeability which was not observed in vivo. Also, the rate of removal of Ni^{2} from the airways was inhibited by the presence of Ca^{2+} and Mg^{2+} in the perfusate. Uptake of Ni^{2} from the vasculature was studied in the IVPL supplied with $NiCl_2$ at 0.01,0.1, 1.0, and 10 µmol/min. The net rate of Ni^{2+} varied with the supply rate and did not appear to be saturable. A two-compartment model described the efflux of Ni^{2+} from the vasculature. These studies suggest that the airways of the mammalian lung are largely impermeable to inhaled Ni^{2+} in the presence of physiologic concentrations of divalent cations, while uptake from the vasculature is relatively rapid.

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The Distributional Kinetics of Ni^{2+} in the Rat Lung[1]

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The Distributional Kinetics of Ni^{2+} in the Rat Lung. WILLIAMS, S. J., HOLDEN, K. M., SABRANSKY, M., AND MENZEL, D. B. (1980). *Toxicol. Appl. Pharmacol.* **55**, 85–93. The kinetics of removal of Ni^{2+} from the airways of the rat lung was studied *in vivo* and *ex vivo*. The uptake of Ni^{2+} from the vasculature was investigated *ex vivo*. For *ex vivo* studies, the isolated, ventilated, and perfused lung (IVPL) was prepared using negative pressure ventilation and was perfused with Krebs–Henseleit buffer solution at 37°C. Rates of removal of Ni^{2+} from the airways were determined following intratracheal instillation of 1, 10, or 127 nmol $^{63}NiCl_2$ in 100 μl of isotonic sucrose. The Ni^{2+} remaining in the lung was calculated from the $^{63}Ni^{2+}$ activity which appeared in the perfusate. *Ex vivo* studies revealed a concentration dependent alteration in lung permeability which was not observed *in vivo*. Also, the rate of removal of Ni^{2+} from the airways was inhibited by the presence of Ca^{2+} and Mg^{2+} in the perfusate. Uptake of Ni^{2+} from the vasculature was studied in the IVPL supplied with $NiCl_2$ at 0.01, 0.1, 1.0, and 10 μmol/min. The net rate of uptake of Ni^{2+} varied with the supply rate and did not appear to be saturable. A two-compartment model described the efflux of Ni^{2+} from the vasculature. These studies suggest that the airways of the mammalian lung are largely impermeable to inhaled Ni^{2+} in the presence of physiologic concentrations of divalent cations, while uptake from the vasculature is relatively rapid.

The presence of nickel bearing compounds in the atmosphere is largely a result of the combustion of fossil fuels by man. Crude oil and coal contain trace quantities of nickel which, upon combustion, are converted to a variety of oxides and sulfides and nickel carbonyl. The atmospheric burden and, ultimately, the quantity of nickel to which humans may be exposed are determined by a number of factors. These include the geographic sources of the fuel, the proximity of the combustion facility to humans, the physical characteristics of the facility, and a variety of atmospheric parameters. Furthermore, the toxicity of these compounds may be greatly influenced by the physical characteristics of the particulate fractions with which they are associated. Natusch *et al.* (1974) have demonstrated that the heavy metal content of fly ash is roughly inversely proportional to the aerodynamic diameter of the particulate fraction. Nickel was found to be concentrated by a factor of 4 in the particles of respirable size (1–2 μm equivalent aerodynamic diameter) compared to larger particles (4–7 μm equivalent aerodynamic diameter). The average annual urban concentration of nickel is about 0.06 $\mu g/m^3$ (Lee *et al.*, 1972), and in certain urban centers the total amount of inhaled nickel could be as high as 2 to 14 μg per person per day (Schroeder, 1970). The quantity of inhaled nickel could be increased significantly by cigarette smoking (Menden *et al.*, 1972) and industrial exposure.

[1] This paper was presented in part on August 17, 1978, in Houston, Tex. at the joint meeting of the American Society for Pharmacology and Experimental Therapeutics and the Society of Toxicology.

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The physical and chemical properties of nickel in the various nickel bearing compounds are the factors which ultimately determine their toxicity. The size, valency, electronegativity, and solubility of the compound determine the extent to which it will form complexes with biological molecules and alter the function or availability of these macromolecules to the detriment of the organism. Most heavy metals are able to combine with sulfhydryl, hydroxyl, carboxyl, imidazole, and amine groups of biomolecules to varying degrees, and these interactions may be responsible for their toxic effects (Luckey and Venugopal, 1977). The efficiency of lung homeostatic mechanisms in clearing or sequestering these compounds may determine to what extent they will manifest toxicity. The data presented in this study demonstrate that the lung may not have an effective clearance mechanism for trace quantities of Ni^{2+} and that accumulation of this compound may occur in pulmonary tissue.

MATERIALS AND METHODS

Male Sprague–Dawley rats (176–200 g) maintained on Purina Lab Chow and water *ad libitum* were used in all experiments.

Removal of Ni^{2+} from the Airways of the Isolated, Ventilated, and Perfused Lung (IVPL)

Rats were anesthetized with sodium pentobarbital (35 mg/kg, ip). The trachea was isolated and a cannula (PE 240) was inserted and sutured in place. A midline incision was made and the major abdominal vessels were severed. After exsanguination, the chest was opened, the left atrium was removed, and the pulmonary artery was cannulated (PE 200). The pulmonary vein was not cannulated. The lungs were removed from the chest cavity and placed in an artificial thorax (37°C). The lungs were then perfused with Krebs–Henseleit buffer containing 4% bovine serum albumin (BSA) (Sigma Chemical Company, St. Louis, Mo.) supplemented with 5 mM glucose and 0.5 mM EDTA and equilibrated with 5% O_2:95% CO_2 at 37°C. Perfusion was begun by gravity feed (25 mm Hg) until the lungs were cleared of blood. The perfusion was then switched to a peristaltic pump in a recirculating system of constant volume (100 ml), and flow was maintained at 10 ml/min. All solutions were filtered through a 0.2-μm membrane prior to use. Pressure in the arterial line was monitored continuously (Statham P23C pressure transducer) for evidence of edema formation (24–26 mm Hg). Respiration was performed under negative pressure (−3 to −15 cm H_2O; V_T, 1.5–2 ml; 80 breaths per minute) with a rodent respirator (Harvard Apparatus, Millis, Mass.) and a vacuum pump. To study the removal of Ni^{2+} from the airway, 100 μl of isotonic sucrose (pH 7.4) containing 1, 10, or 127 nmol $^{63}NiCl_2$ (New England Nuclear, Boston, Mass.) was instilled intratracheally at the point of maximum expiration (−3 cm H_2O). Aliquots of the perfusate (100 μl) were taken from the stirred reservoir every 5 min for 90 min and assayed for ^{63}Ni activity by liquid scintillation spectrometry (LS-100, Beckman Instruments, Norcross, Ga.). Lungs were weighed at the termination of each experiment and dessicated over sodium hydroxide under vacuum until a constant dry weight was achieved.

Removal of Ni^{2+} from the Airways in Vivo

A modification of the technique of Enna and Shanker (1972) was employed in these studies. Rats were anesthetized with sodium pentobarbital (35 mg/kg, ip) and secured on their backs on a dissecting tray. The tracheae were cannulated and the animals were supported vertically. Isotonic sucrose (100 μl) containing the various quantities of $^{63}NiCl_2$ was instilled intratracheally. The wounds were covered with sterile gauze saturated with Krebs solution; the rats were then returned to a normal resting position and maintained under light anesthesia for the requisite time. One minute before the end of the experimental period, surgical removal of the lungs was initiated. Animals were exsanguinated; and upon removal, the whole lung was digested in 10 ml of 60% $HClO_4$ plus 5 ml of 30% H_2O_2 at 85°C (Mahin and Lofberg, 1966). After digestion was complete, the solution was neutralized with 7.5 ml of 15.5 N NaOH and diluted to 25 ml. Aliquots were assayed for ^{63}Ni activity using an internal standard and corrected for percentage recovery at the given dose.

Uptake of Ni^{2+} from the Vasculature of the IVPL

The IVPL was prepared as described above. However, the Krebs–Henseleit perfusate was prepared without BSA or disodium EDTA to prevent interference of these compounds with the activity of Ni^{2+} at the luminal surface of the capillary. The calcium concentration was reduced by 0.5 mM to account for the removal of the EDTA. When all blood was washed from the lung, the perfusion was switched to a second-

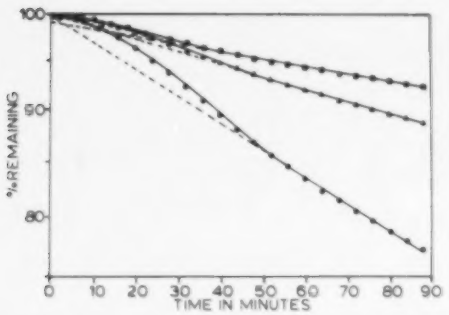

FIG. 1. Removal of $^{63}Ni^{2+}$ from the airways of the rat lung measured *ex vivo* following intratracheal instillation of 1 (■), 10 (▲), or 127 (●) nmol of Ni^{2+} per lung. The rates of removal were calculated from the slope of the terminal region of the curve, as indicated by the dashed line.

ary vessel in a flow-through system containing Blue Dextran 2000 (5 mg/ml) as a vascular marker and $NiCl_2$ (5, 50, 500, or 5000 nmol/ml) in Krebs–Henseleit buffer. The flow rate was maintained at 2 ml/min to prevent edema formation in the absence of BSA. Perfusion was continued for 10 min with this solution, and the lungs were washed for an additional 10 min. Fractions of the venous effluent were collected every 0.2 min, diluted to 3.0 ml, and assayed for ^{63}Ni activity and blue dextran concentrations at 600 nm by spectrophotometry. Wet and dry weight determinations were made as previously described.

The unidirectional rate of uptake of Ni^{2+} from the vasculature was determined according to a modification of the method of Pickett *et al.* (1975) by calculating the venous concentration of Ni^{2+} at the point of equal perfusion of blue dextran. The point of equal perfusion was determined from the first time point where no arterial to venous difference of the blue dextran concentration existed. The rate of uptake of Ni^{2+} was calculated from the formula:

$$\text{rate of uptake} = \frac{\text{effluent sample volume}}{\text{duration of sample collection}} \times (\text{arterial conc} - \text{venous conc})$$

RESULTS

Removal of Ni^{2+} from the Airways ex Vivo

Figure 1 demonstrates that the removal of Ni^{2+} from the airways of the isolated, perfused lung is predominantly by a process of diffusion across the membranes of the lung parenchyma and vasculature. There are, however, under lying complexities in the removal process. It is apparent from Fig. 1 that the rate of removal of Ni^{2+} from the airways is a dose-dependent phenomenon. The effect is also time related and appears to require at least 30 min before equilibration is reached. Therefore, the first-order rate constants for the removal of Ni^{2+} from the airways (Table 1) were calculated from the terminal portions (40–90 min) of the efflux curves which were linear. The rate constant of removal was found to be directly proportional to the log of the dose given intratracheally.

The influence of physiological cationic species on the removal of Ni^{2+} from the airways is shown in Fig. 2 and Table 2. The concentrations of Mg^{2+} and Ca^{2+} in the perfusate profoundly inhibit the ability of Ni^{2+} to cross the membranes of the lung and reach the vascular space. Removing either or both of these ions from the perfusate increases the removal rate of Ni^{2+} proportionally to the chemical activity of the ions in the perfusate under control conditions. (For example, Table 2 shows that in the absence of Ca^{2+}, the rate of removal of Ni^{2+} from the airways is twice as fast as it is in the

TABLE 1

RATE OF ABSORPTION OF $^{63}Ni^{2+}$ FROM THE AIRWAYS OF THE ISOLATED, VENTILATED, AND PERFUSED RAT LUNG[a]

Total dose per lung (nmol)	Rate constant of removal ($\times 10^{-3}$ min^{-1})	$T_{1/2}$ (hr)
1	0.71 ± 0.17[b]	19.77 ± 6.49
10	1.31 ± 0.20[c]	9.21 ± 1.27
127	2.40 ± 0.17[d]	4.58 ± 0.34

[a] Each value represents the mean ± SE of at least four animals.

[b] $p \leq 0.05$ compared to 10-nmol dose by the Student's t test.

[c] $p \leq 0.01$ compared to 127-nmol dose by the Student's t test.

[d] $p \leq 0.001$ compared to 1-nmol dose by the Student's t test.

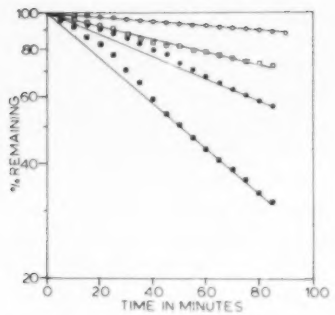

FIG. 2. The influence of the divalent cationic strength of the perfusate upon the removal of 10 nmol $^{63}Ni^{2+}$ from the airways of the rat lung *ex vivo*. The perfusate used was Krebs–Henseleit (○), or Krebs–Henseleit depleted of Mg^{2+} (□), Ca^{2+} (●), or Ca^{2+} and Mg^{2+} (■).

absence of Mg^{2+}. The approximate ratio of the concentrations of these ionic species is 2:1 in Krebs–Henseleit (2.5 mM Ca^{2+}:1.2 mM Mg^{2+}).)

Removal of Ni^{2+} from the Airways in Vivo

The first-order rate constant for the removal of Ni^{2+} from the airways of the rat lung *in vivo* is independent of the dose of nickel instilled (Fig. 3). These data provide further support for the hypothesis that the removal of Ni^{2+} from the airways of the rat lung is by a process of diffusion. The values obtained for these rates compare favorably to the results obtained *ex vivo*. However, this technique does not appear to be sensitive enough to detect dose related changes in removal rates as were observed *ex vivo*.

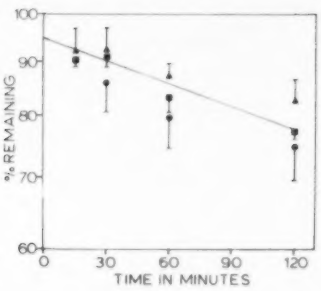

FIG. 3. Removal of $^{63}Ni^{2+}$ from the airways of the rat lung *in vivo* following intratracheal instillation of 1 (■), 10 (▲), or 127 (●) nmol of Ni^{2+} per lung. Each point represents the mean ± SE of at least three animals. The slopes of the lines through each set of data did not differ significantly when calculated by the method of least squares. The solid line was calculated by a linear regression program which included all values obtained. The slope of this line is equivalent to the mean removal rate at all doses ($m = 1.75 \pm 0.27 \times 10^{-3}$ min^{-1}, $r^2 = 0.74$).

TABLE 2

EFFECT OF CATIONIC CONCENTRATION ON REMOVAL OF 10 nmol $^{63}Ni^{2+}$ FROM THE AIRWAYS OF THE ISOLATED, VENTILATED, AND PERFUSED RAT LUNG[a]

Perfusate	Rate constant of removal ($\times 10^{-3}$ min^{-1})
Control (1.2 mM Mg^{2+}, 2.5 mM Ca^{2+}; μ = 316 mOSM/liter)	1.31 ± 0.20[b]
Mg^{2+} and Ca^{2+} depleted (3.7 mM sucrose; μ = 316 mOSM/liter)	16.8 ± 2.6[b]
Ca^{2+} depleted (1.2 mM Mg^{2+}, 2.5 mM sucrose; μ = 316 mOSM/liter)	6.78 ± 0.67[b]
Mg^{2+} depleted (2.5 mM Ca^{2+}, 1.2 mM sucrose; μ = 316 mOSM/liter)	3.70 ± 0.13[b]

[a] The ionic composition of the Krebs–Henseleit perfusate was altered as indicated, but the total osmolarity of the solution was held constant. Each value represents the mean ± SE of at least four animals.

[b] All values were significantly different at the $p \leq 0.01$ level when evaluated by Student's t test.

Uptake of Ni^{2+} by the Lung ex Vivo

A graph of the vascular uptake data (Fig. 4) shows Ni^{2+} to have an apparent volume of distribution greater than that of blue dextran, a compound which remains in the vascular space. Nickel is removed from the vasculature by a diffusional process, since the net rate of uptake is proportional to the concentration of Ni^{2+} infused (Table 3 and Fig. 5). A significant arterial to venous concentration difference of Ni^{2+} was observed at all concentrations tested (Table 3 and Fig. 4). The extraction ratio is independent of the supply rate, suggesting that the portion of

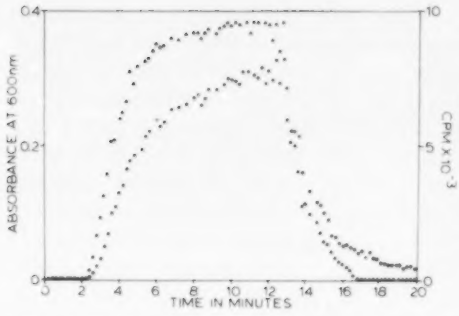

FIG. 4. Uptake of $^{63}Ni^{2+}$ from the vasculature of the rat lung *ex vivo*. Blue dextran (△) (5 mg/ml) and Ni^{2+} (○) (0.01, 0.1, 1.0, or 10 μmol/min) were infused concomitantly and measured in the venous effluent. Equal perfusion of the lung was assumed to occur at the point at which the blue dextran concentration in the venous effluent was equal to the arterial concentration. In this plot, Ni^{2+} was infused at a rate of 1 μmol/min. Infusion of Ni^{2+} was begun at time zero and was terminated at 10 min. The dead volume in the system was approximately 4 ml.

the infused nickel which was taken up may be sequestered in some unsaturable pool in the lung. The addition of disodium EDTA (0.5 mM) to the perfusate totally abolished the uptake of nickel from the vasculature as well as the arterial–venous difference. Thus, in the presence of EDTA, a chelator of metal ions, Ni^{2+} exhibited kinetics which paralleled those of the vascular marker.

Diffusional kinetics across the vascular membranes are further corroborated by nonlinear regression analysis of the washout curve for infused Ni^{2+} (Table 3 and Fig. 6). This curve is resolvable into two exponential components. The slope of the fast component is parallel to the slope of the efflux curve for blue dextran. This component, then, represents the removal of Ni^{2+} from the vascular lumen. The slow component of the curve is representative of the unidirectional flux of Ni^{2+} from some extravascular space. If the net rates of uptake are expressed as fractions of the supply rates (i.e., as first-order rate constants), it is apparent that these rates do not differ significantly from the removal rates calculated at supply rates of 0.01, 0.1, and 1.0 μmol/min. This suggests that the unidirectional rates of flux being measured by these two methods represent diffusion of Ni^{2+} across the same membrane. The rate constant of removal was significantly lower at the highest Ni^{2+} concentration. Thus, at this high dose Ni^{2+} may reach areas of the lung where it can be sequestered more tenaciously than at lower doses.

Dry Weight to Wet Weight Ratios

In order to determine the extent of edema formation in the isolated lung preparation as it may affect results, dry to wet weight ratios were determined for all perfused lungs. Lungs perfused with Krebs–Henseleit solution plus 4% BSA for 90 min were used as controls and had a dry to wet weight ratio of 17.85 ± 0.33% (n = 3). Only under ex-

TABLE 3

VASCULAR UPTAKE OF $^{63}Ni^{2+}$ BY THE ISOLATED, VENTILATED, AND PERFUSED RAT LUNG[a]

Supply rate (μmol × min^{-1})	Net rate of uptake[b] (μmol × 10^{-3} min^{-1})	Rate constant of removal[c] ($\times 10^{-1}$ min^{-1})	Extraction ratio[d]
0.01	3.96 ± 0.29	1.71 ± 0.11	32.0 ± 2.1
0.10	38.7 ± 5.8	2.36 ± 0.21	26.6 ± 5.6
1.00	266 ± 22	2.59 ± 0.34	20.3 ± 1.33
10.0	2700 ± 590	0.66 ± 0.09	24.0 ± 5.1

[a] Results are the mean ± SE of at least three animals at each dose.

[b] The net rate of uptake of $^{63}Ni^{2+}$ was calculated at the point of equal perfusion of the lungs as indicated by the blue dextran concentration in the venous effluent.

[c] Rate of removal of $^{63}Ni^{2+}$ from the vasculature was calculated as described in the text and as illustrated in Fig. 6.

[d] The venous concentration of $^{63}Ni^{2+}$ was calculated from the plateau region of the uptake curve, and the extraction ratio was obtained from the equation

$$\frac{\text{arterial concentration} - \text{venous concentration}}{\text{arterial concentration}} \times 100.$$

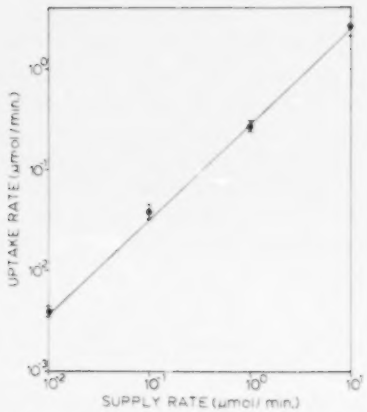

FIG. 5. Uptake rate of $^{63}Ni^{2+}$ from the vasculature of the rat lung calculated at the point of equal perfusion at various supply rates. Each point represents the mean ± SE of at least three animals.

perimental conditions in which BSA was absent from the perfusate did this ratio deviate significantly from controls. In these cases a ratio of 16.82 ± 0.20% (n = 12) was found. If the ratio was less than 16%, the experimental data were not used.

DISCUSSION

Given the current lack of knowledge concerning the oxidative state of nickel in the atmosphere, Ni^{2+} serves as a good model for inhalation studies concerning the toxicity of nickel bearing compounds which may be present in atmospheric particulates. Differences in pulmonary pathology induced by various nickel compounds may be related to differences in their solubility in the lung. *In vitro* hierarchical classifications of compound solubilities may not apply when these compounds are inhaled, however. An example of this is the rapid solubilization of cadmium oxide in the lung (Hadley *et al.*, 1978). Also, $Ni(CO)_4$ is more toxic than an equivalent dose of $NiCl_2$ due to the high lipid solubility of the former compound and, hence, its ability to reach internal cellular structures and release Ni^{2+}.

Whole body distributional studies following the injection of $NiCl_2$ and $Ni(CO)_4$ in mice suggest that the lungs may be a target tissue for the toxicity of inorganic and organic nickel compounds (Oskarrson and Tjälve, 1977, 1979). In mice injected ip with $^{63}NiCl_2$, autoradiography showed that the highest density of radioactivity was present in lung tissue of animals sacrificed 5 days or more postinjection. Calcium was able to displace the radioactivity from cartilage more easily than from lung tissue. If mice were injected with $^{63}Ni(CO)_4$, high radioactivity was present in the lungs at all survival times. If, on the other hand, $Ni(^{14}CO)_4$ were injected, most of the radioactivity was present in the blood. These results suggest that a tenacious chemical interaction of Ni^{2+} with lung tissue may occur and that Ni^{2+} may be the toxic metabolite of $Ni(CO)_4$.

The binding of Ni^{2+} to a wide variety of macromolecules has been investigated and may be a major determinant in chronic toxicity, carcinogenicity, and alterations in membrane permeability induced by a variety of nickel bearing compounds. The

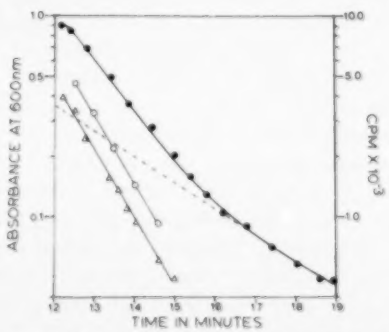

FIG. 6. Efflux of $^{63}Ni^{2+}$ from the vasculature of the rat lung *ex vivo*. The activity of $^{63}Ni^{2+}$ (●) and the absorbance of blue dextran (△) were plotted semilogarithmically. A regression program (HP 9825A; Hewlett–Packard Instrument Company, Loveland, Colo.) was employed to make a linear transformation of the general first order equation, $y = ae^{-bx}$ (– – –), which was subtracted from the Ni^{2+} efflux curve (●). The resultant (○) had a slope equal to that of the efflux curve for blue dextran.

binding of nickel to serum protein has been implicated as a critical reaction in the development of nickel hypersensitivity (Cotton, 1964). Sunderman (1963) has demonstrated specific binding of ^{63}Ni to rat lung RNA following exposure of rats to $^{63}Ni(CO)_4$. The reaction of nickel ions with a wide variety of biological materials may result in drastic alteration in membrane and cell structure and function. The ability of nickel to induce cancer in laboratory animals and in nickel refinery workers (Doll, 1958) is probably related to these types of molecular interactions, although a specific mechanism of carcinogenesis has not been established. Similar, but comparatively nonspecific, molecular interactions of nickel may explain the complex nature of the kinetic data presented here.

The toxic effects of Ni^{2+} at the cell membrane may be related to induced alterations of membrane permeability. The inhibition of Na^+, K^+ ATPase activity in rat brain capillary membranes is probably related to changes in the permeability of these structures (Joó, 1969). Also, $NiCl_2$ has been shown to enhance the diffusion of SO_4^{2-} from the rat by as much as 30%, whether given simultaneously with the nickel by intratracheal instillation or given intratracheally following exposure of rats to an aerosol of $NiCl_2$ (Charles *et al.*, 1977). Sulfate, however, does not affect the rate of removal of Ni^{2+} from the rat lung (Williams and Menzel, 1979). The results presented here are suggestive of nickel-induced permeability changes in the lung epithelium, since the rate of removal is proportional to the dose instilled intratracheally. The first-order removal rate of intratracheally instilled Co^{2+} from the airways is not dose dependent over the same concentration range (Williams *et al.*, 1979), so these kinetics are probably not a result of the increased chemical gradient of Ni^{2+} across the membrane at higher doses.

Our results indicate that the passage of Ni^{2+} across the lung epithelium is a relatively slow and complex process. Co^{2+}, for example, exhibits an initial first-order rate constant of removal of $8.61 \pm 1.02 \times 10^{-3}$/min which is independent of the dose (Williams *et al.*, 1979). Sulfate ion is removed at a rate two orders of magnitude faster than nickel at equimolar doses (Charles *et al.*, 1977). Also, the rates measured here can be considered only as initial rates of removal. It is likely that at longer time periods the rate of removal would become dependent on the rate of turnover of the various molecular fractions in the lung with which bound nickel is associated. While these experiments do not delineate a specific site of absorption in the lung, the similarity between the results obtained *in vivo* and *ex vivo* rules out significant removal by the tracheobronchial circulation or by the lymphatic system, both of which are severed in the latter preparation. The alveolar macrophage would not be expected to play an important role in this removal process, given the relatively short period of exposure. Also, macrophages are most efficient at clearing the respiratory regions of the lung of particulates and have not been shown to have a higher affinity for soluble Ni^{2+} than any other pulmonary cell type. Furthermore, Ni^{2+}, at a concentration of 500 μM, has been shown to reduce the phagocytic activity of the murine alveolar macrophage by 50% *in vitro* (Graham *et al.*, 1975). Our lungs have a total water content of about 1.2 ml. Thus, a 100-nmol dose of Ni^{2+} would represent a concentration of 80 μM, assuming equal distribution of Ni^{2+} in lung water, and could result in significant inhibition of macrophage activity.

The results obtained by altering the Mg^{2+} and Ca^{2+} concentrations of the perfusate are evidence that Ni^{2+} shares a common site of translocation with these ions in lung membranes. A competition between Ni^{2+} and Ca^{2+} for channels and binding sites has been observed in a variety of tissues. In frog skeletal muscle, Ni^{2+} and Ca^{2+} fluxes are quite similar in the resting state, but Ni^{2+}

flux does not increase when the muscle is stimulated to contract (Fischner and Sevan, 1976). At the neuromuscular junction, Ni^{2+} competes with Ca^{2+} and decreases the number of quanta released per impulse (Benoit and Mambrini, 1970). At these sites, as in the lung, Ni^{2+} and physiological divalent cations may exhibit similar kinetic behavior, while the biochemical actions of these ions may be quite different.

The sequestration and binding of Ni^{2+} to lung tissues are suggested by the results obtained from the vascular uptake studies. The large arterial to venous difference is evidence that Ni^{2+} passes freely from the vascular space and is sequestered in the lung. The efflux curve shows that very little of the Ni^{2+} taken up by the lung is recovered in the venous washout. In comparison, sulfate exhibits the same pattern of distribution as the vascular marker when infused into the isolated, perfused rat lung and is totally recovered in the venous effluent (Charles and Menzel, 1976). Sulfate has not been shown to bind strongly to any biological macromolecule.

The diversity of nickel compounds, routes of administration, and species tested to date make evaluation of their relative toxicity and carcinogenic potential difficult. However, parenteral administration of nickel compounds increases toxicity by at least one order of magnitude over oral toxicity (National Academy of Sciences, 1975). This may be related to the negligible intestinal absorption of ingested nickel, since approximately 90% of an oral dose of $NiCl_2$ is excreted in the feces (Tedeschi and Sunderman, 1957) and parenterally administered Ni^{2+} is largely excreted in the urine (Onkelinx, 1973).

It is apparent from our studies that the slow removal of Ni^{2+} from the airways may result in its accumulation in the lung during chronic exposure to low levels of atmospheric nickel. The lung may also have a high capacity to sequester tenaciously significant quantities of this compound. These data represent the combined kinetic contribution of at least 16 different cell types in the rat lung. Ni^{2+} may have unique kinetic characteristics in each of these. Type II epithelial cells, for example, appear to be quite permeable to Ni^{2+} (Saito and Menzel, 1978) and exhibit multiple flux constants. Therefore, rapid uptake by specific cell types, combined with the slow removal of Ni^{2+} from the lung as a whole, may lead to an effective increase in the exposure time of lung cells to the compound. Chances for alterations in cellular regulation and function at the membrane and intracellular levels are, thus, improved.

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